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European Patent Office
Office européen des brevets



(11) Publication number:

0 594 847 A1

EUROPEAN PATENT APPLICATION
published in accordance with Art.
158(3) EPC

(21) Application number: 91912352.1

(22) Date of filing: 11.07.91

(96) International application number:
PCT/JP91/00931

(87) International publication number:
WO 92/01052 (23.01.92 92/03)

(51) Int. Cl.⁵: C12N 15/61, C12N 9/90,
C12N 1/21, //(C12N1/21,
C12R1:19)

(30) Priority: 11.07.90 JP 183238/90

(43) Date of publication of application:
04.05.94 Bulletin 94/18

(84) Designated Contracting States:
CH DE FR GB LI

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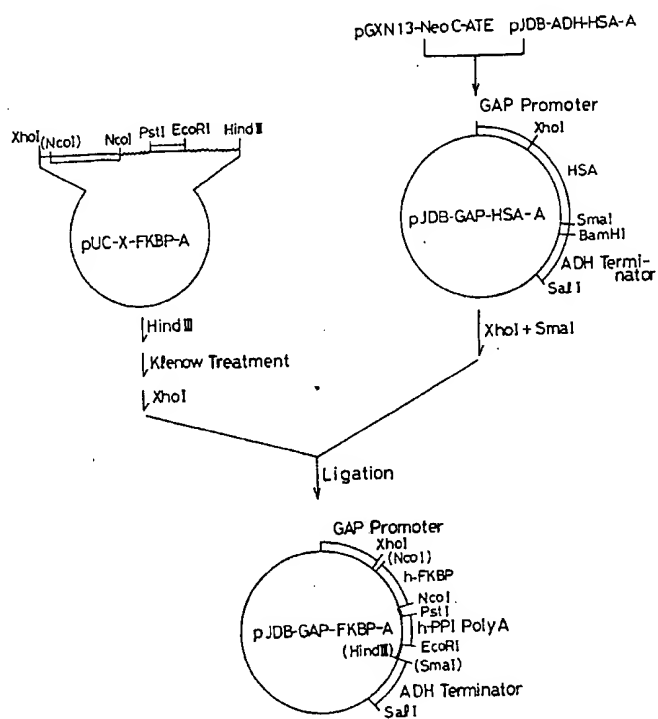
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(54) DNA CODING FOR HUMAN FK506-BINDING PROTEIN AND EXPRESSION THEREOF.

(57) A DNA containing a base sequence which codes for a human FK506-binding protein composed of 108 amino acid residues; a recombinant human FK506-binding protein prepared by the expression of the DNA; and an expression vector for use in the expression and a transformant. The invention serves to establish a process for mass-producing a human FK506-binding protein and realizes a stabilized supply of a human FK506-binding protein having a peptidylprolyl cis-trans isomerase activity for screening immunosuppressants.

Fig. 2



This invention relates to a DNA molecule coding for human FK506-binding protein and to expression thereof.

Background of the Invention

5 Peptidyl prolyl cis-trans isomerase (referred to as "PPlase" hereinafter) is known as the enzyme which catalyzes isomerization of a proline-containing peptide linkage and promotes formation of the higher-order structure of a protein. This enzyme has been proved to be identical to cyclophilin which binds specifically to an immunosuppressive agent, cyclosporin A, (Takahashi, N. *et al.*, *Nature*, vol.337, pp.473 - 475, 1989; 10 Lang, K. *et al.*, *Nature*, vol.329, pp.268 - 270, 1987). PPlase is also capable of promoting folding of the higher-order structure of denatured proteins such as ribonuclease A, ribonuclease T₁, immunoglobulin, cytochrome C and type III collagen (Fischer, G. and Bang, H., *Biochim. Biophys. Acta*, vol.828, pp.39 - 42, 1985; Lin, L.-N. *et al.*, *Biochim. Biophys. Acta*, vol.956, pp.256 - 266, 1989; Bachinger, H.P., *J. Biol. Chem.*, vol.262, pp.17144 - 17148, 1987).

15 From the fact that PPlase activity of cyclophilin is inhibited by the immunosuppressive agent cyclosporin A, such an effect of cyclosporin A has been considered to be achieved by inhibiting the activity of PPlase and thus repressing exhibition of the activity of a substrate protein for PPlase. However, as described below, it has been suggested that there exist a number of protein species as substrate for cyclophilin, as well as a variety of effects of cyclosporin A. For example, cyclosporin A activates the helper 20 T cell among cells involved in the immune system, and cyclophilin serves as PPlase and is involved in the activation of certain transcriptional control factors, such as NFAT and AP-3, which induce expression of interleukin-2 gene and other lymphokine genes (Takahashi N. *et al.*, *Gendai Kagaku (Japan)*, vol.222, pp.40 - 47, 1989; Emmel, E.A. *et al.*, *Science*, vol.246, pp.1617 - 1620, 1989). Also, cyclosporin A inhibits production and secretion of γ -interferon, interleukin-4, macrophage-activating factor, tumor necrosis factor 25 and the like in immune cells, and cyclophilin probably serves as PPlase in the transcription, translation, or formation of higher-order structures of those proteins (Takahashi N., *Igaku No Ayumi (Japan)*, vol.151, pp.413 - 416, 1989).

In mammals, cyclophilin has been identified as one type of the protein per one biospecies. However, various types of cyclophilin-like proteins are now believed to exist in mammals because it has been found 30 that there are several tens of DNA copies encoding cyclophilin-like proteins on mammalian chromosomal DNA (Haendler, B. *et al.*, *EMBO J.*, vol.6, pp.947 - 950, 1987) and because two different types of cyclophilin-like proteins have been found in yeast and *Escherichia coli* (Japanese Patent Application No. 1-184738 and Japanese Patent Application No. 1-344705). Such a diversity is correlated with the variety of the related substrate proteins, and this may be the cause of the side effects of cyclosporin A when used as 35 a drug.

FK506 is also known as the immunosuppressive agent which has quite similar effects to those of cyclosporin A but has a completely different chemical structure therefrom (Thomson, A.W., *Immunology Today*, vol.10, pp.6 - 9, 1989). In addition, it is known that a binding protein having the specific affinity to FK506 *in vivo* exists and that the FK506-binding protein has PPlase activity which is inhibited by binding of 40 FK506 (Siekierka, J.J. *et al.*, *Nature*, vol.341, pp.755 - 757, 1989; Harding, M.W. *et al.*, *Nature*, vol.341, pp.758 - 760, 1989).

The fact that these two immunosuppressive agents, i.e., cyclosporin A and FK506, have the same function to inhibit PPlase activity, in spite of their completely different chemical structures, strongly suggests that immunosuppression is mediated through the inhibition of PPlase activity. In other words, any 45 substance capable of inhibiting PPlase activity may possess immuno suppressive activity.

From the comparative studies on the substrate specificities of FK506-binding protein and cyclophilin based upon their PPlase activities, it has been found that cyclophilin has a relatively wide range of substrate specificity while FK506-binding protein has a narrower range of specificity than cyclophilin (Harrison, R.K. and Stein, R.L., *Biochemistry*, vol.29, pp.3813 - 3816, 1990). These suggest that substrates for FK506- 50 binding protein may be different from those for cyclophilin, and FK506-binding protein acts with higher specificity compared with cyclophilin.

An immunosuppressive agent that can act selectively to immune-system cells and, especially, can specifically inhibit PPlase activity in helper T cells is expected to be a useful active agent having less side effect, and any immunosuppressive agent having a low or less side effect can be screened based upon the 55 activity for inhibiting FK506-binding protein that has a narrower range of substrate specificity than cyclophilin.

FK506-binding protein has been isolated directly from natural sources by two research groups, but the reported molecular weights thereof are different from each other: 11,000 daltons as reported by Siekierka,

J.J. *et al.* (*Nature*, vol.341, pp.755 - 757, 1989) and 14,000 daltons as reported by Harding, M.W. *et al.* (*Nature*, vol.341, pp.758 - 760, 1989). In the latter reference, Harding *et al.* have determined the partial amino acid sequence (up to 40 residues of the amino terminal region) of the protein from bovine thymus, and concluded that the isolated protein is different from cyclophilin, though it is not clear whether said

5 protein has any commonness with cyclophilin in terms of the active site of PPlase.
As aforesaid, the FK506-binding protein has a different PPlase activity from that of cyclophilin and it seems to have various physiological functions. This binding protein is regarded as the important material which will be used as a means for screening any immunosuppressive agent based on the inhibition of its PPlase activity, and as a means for promoting *in vitro* formation of the higher-order structure of a specific

10 protein, as well as its use as a reagent for the examination of substrate specificity of the binding protein. Process for the manufacture of FK506-binding protein, however, has not been established except its direct isolation from mammalian organs.
Accordingly, an object of the present invention is to determine a DNA sequence coding for the FK506-binding protein and an amino acid sequence deduced from the DNA sequence, by isolating the gene that

15 encodes the binding protein.
Another object of the present invention is to provide a process for manufacturing the FK506-binding protein on a large scale using genetic engineering techniques.

Summary of the Invention

20 The present invention provides a DNA molecule comprising a base sequence coding for the human FK506-binding protein which is represented by the amino acid sequence of position 1 - position 108 of SEQ ID NO:1.

A gene encoding the human FK506-binding protein can be obtained by plaque hybridization from a

25 human T cell cDNA library supplied from CLONTECH Inc. (U.S.A.), which has been prepared using λ gt 11 as a vector. Molecular weight of the human FK506-binding protein is 11,951 daltons as calculated based on its deduced amino acid sequence, and its isoelectric point is 8.71. The nucleotide sequence of this gene is completely novel compared to the registered data bases and has no homology with that of cyclophilin having a PPlase activity.

30 The present invention also provides an expression vector containing said DNA molecule and a transformant obtained by introducing the expression vector into a host cell.
In addition, the present invention provides a process for the manufacture of a recombinant human FK506-binding protein, which comprises expressing said vector through a culture of said transformant, as well as the recombinant human FK506-binding protein obtained.

Brief Description of the Drawings

Fig. 1 shows the construction of the plasmid pUC-X-FKBP-A which contains a DNA fragment coding for the human FK506-binding protein.

40 Fig. 2 shows the construction of the plasmid pJDB-GAP-FKBP-A.

Detailed Description of the Invention

The present invention provides a DNA molecule comprising a base sequence coding for human FK506-binding protein which is represented by the amino acid sequence of position 1 - position 108 of SEQ ID NO:1.

The followings describe isolation, sequencing, and expression of the cDNA concerning this invention:

Isolation and sequencing of cDNA

50 A probe to be used can be synthesized based on the amino acid sequence (from Glu at position 31 to Asp at position 37) of bovine FK506-binding protein (referred to as "FKBP" hereinafter) which has been reported by Harding *et al.* (*Nature*, vol.341, pp.758 - 760, 1989). Using the synthetic probe, a positive clone containing human FKBP cDNA, which has been transfected into an *Escherichia coli* strain, can be

55 screened by hybridization from the human T cell cDNA library which has been prepared by CLONTECH Inc.(U.S.A.), using λ gt 11 as a vector.

The positive clone is lysed to obtain phage particles from which phage DNA is recovered by conventional phenol/chloroform extraction and ethanol precipitation. After digesting the phage DNA with

EcoRI in a buffer solution containing RNase A, the resulting fragments are subjected to agarose gel electrophoresis and Southern blotting (Southern, E.J., *J. Mol. Biol.*, vol.98, pp.503 - 517, 1975). A clone containing an *EcoRI* fragment of about 1.5 kb in length is obtained by repeating the hybridization step, and the *EcoRI* fragment is isolated and purified from the agarose gel by a conventional technique such as the glass powder method (Gene Clean™, Bio-101).

Next, the 1.5 kb *EcoRI* fragment obtained is inserted into an *EcoRI*-digested vector pUC 19 using T4 DNA ligase in order to construct a recombinant plasmid. The recombinant plasmid is then transferred into a competent *E. coli* JM 107 strain by the calcium chloride method (Mandel, M. and Higa, A., *J. Mol. Biol.*, vol.53, pp.159 - 162, 1979). A transformant, pUC-h-FKBP, can be obtained by screening ampicillin resistant colonies from the transformed cells. Insertion of the 1.5 kb *EcoRI* fragment into the pUC vector can be confirmed by performing the Mini-Prep of plasmid DNA in the standard way (Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, 1982), digesting the plasmid DNA with *EcoRI*, and then subjecting the resulting fragments to agarose gel electrophoresis. The binding ability of the fragment to said probe can also be ascertained by Southern blotting.

The plasmid DNA obtained is double-digested with *EcoRI* and *HincII* at the *HincII* recognition site located in the DNA insert, and each of the two DNA fragments formed (about 750 bp each) is re-inserted into M13mp phage DNA to determine their nucleotide sequences by dideoxy method (Sanger, F., Nicklen, S. and Coulson, A.R., *Proc. Natl. Acad. Sci. USA*, vol.74, pp.5463 - 5467, 1977). In this way, cDNA which encodes human FKBP can be identified.

Both the nucleotide sequence of the full length human FKBP cDNA and the deduced amino acid sequence therefrom are shown in SEQ ID NO:1 of the Sequence Listing. This gene encodes the protein consisting of 108 amino acid residues (Met Gly ... Leu 108 Glu), as demonstrated by the fact that the above amino acid sequence of human FKBP coincides with 40 amino acid residues from the N-terminus of bovine FKBP which have been reported by Harding *et al.* (*Nature*, vol.341, pp.758 - 760, 1989).

According to an embodiment of the present invention, a base sequence coding for the human FK506-binding protein includes the nucleotide sequence of position 79 - position 402 (7⁹ATG GGA ... CTG 402GAA) shown in SEQ ID NO:1. Any other base sequence, based on the degeneracy of genetic code, which comprises different codons for the same amino acids is also included within the scope of the present invention.

Characteristics of human FKBP

Molecular weight and isoelectric point of human FKBP are 11,951 daltons and 8.71, respectively, as calculated from the deduced amino acid sequence. These data coincides with the molecular weight reported by Harding *et al.* (*Nature* Vol.341, pp.758 - 760, 1989) and with the isoelectric point by Siekierka *et al.* (*Nature*, vol.341, pp.755 - 757, 1989), and thus this indicates that the gene obtained encodes human FKBP. The base and amino acid sequences determined were compared with those registered in data bases (Gen Bank, EMBL, DDBJ), but no homologous gene or protein could be found. Also, there was no homology between human FKBP and cyclophilin when compared to the sequence of cyclophilin which has a PPlase activity (Example 2 herein and Haendler *et al.*, *ENBO J.*, vol.6, pp.947 - 950, 1987). This reveals therefore that the structure of FKBP are completely different from that of cyclophilin. Such a structural difference indicates that these two PPlases probably have different substrate specificity from each other.

When the existence of FKBP-specific mRNA in some organs including lungs, placenta, liver and brain, and in leucocytes was examined by Northern blotting using the isolated gene as a probe, the mRNA was found in all organs and leucocytes tested though the mRNA's content was different among them. Therefore, this means that FKBP is not present tissue-specifically in mammals. When the number of copies of particular genes on human chromosome was examined by Southern blotting, only 1 or 2 copies were detected for the FKBP gene while 20 or more copies have been reported for the cyclophilin gene. These results show that cyclophilin has a variety of molecular species, while the function of FKBP is markedly limited because FKBP has no such a variety. Thus, protein substrates to which FKBP can act are presumed to be relatively limited, because in respect of FKBP the number of molecular species is small and a range of the substrate specificity based upon PPlase activity is very narrow (Harrison, R.K. and Stein, R.L., *Biochemistry*, vol.29, pp.3813 - 3816, 1990). If the protein substrate of FKBP has a tissue-specific function, a substance which can inhibit the FKBP activity probably works tissue-specifically when used as a drug, whereas the inhibitor as an active agent may often give a side effect if it is a common and ubiquitous protein in mammalian tissues. Therefore, if FKBP is easily available, not only this protein can be used as a useful means for screening a substrate of FKBP, but also it will take an important role in the screening of FKBP inhibitors such as an immunosuppressive agent having negligible or less side effect.

Expression of human FK506-binding protein

The present invention also provides an expression vector containing the DNA molecule which comprises a base sequence coding for the human FKBP represented by the amino acid sequence of position 1 - position 108 of SEQ ID NO:1, said DNA molecule being introduced into a cloning site downstream from a promoter and upstream from a terminator in the vector.

Construction of expression vector:

Any known plasmid or phage may be used as a vector. The promoter to be used may be selected from various promoters originated from *E. coli*, phages, eukaryotes such as yeast, and the like. The exemplified promoters include tryptophan operon (*trp*), lactose operon (*lac*), lambda phage P_L or P_R , glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alcohol dehydrogenase I (ADH I) promoters, and the like. Examples of the terminator are terminators of GAPDH gene, ADH I gene and the like.

Since the human FKBP clone prepared as aforesaid does not contain a poly A sequence at its 3' end, the plasmid in which the FKBP structural gene is ligated with a poly A sequence from human cyclophilin cDNA is firstly constructed in accordance with the procedure shown in Fig. 1.

The plasmid pUC-h-FKBP DNA containing FKBP cDNA is digested with *Nco*I and then subjected to the treatment with Klenow fragment to obtain a 340 bp DNA fragment in which the *Nco*I site is blunt-ended. The DNA fragment obtained is purified using Gene Clean™. Separately from this, the cloning vector pUC-18-X in which an *Xho*I site is introduced into the *Eco*RI site of pUC 18 is double-digested with *Eco*RI and *Sma*I and then subjected to the treatment with Klenow fragment to obtain a vector having blunt ends. Thereafter, the vector obtained is ligated with said 340 bp DNA fragment in the presence of T4 DNA ligase to isolate the recombinant plasmid pUC-X-FKBP.

The plasmid pUC-h-PPI DNA containing human PPIase gene is double-digested with *Eco*RI and *Pst*I to obtain a DNA fragment containing a human PPIase poly A sequence of about 150 bp.

The pUC-18-X DNA is also double-digested with *Eco*RI and *Xho*I to obtain a vector fragment of 2.6 kb.

These three DNA fragments prepared above are subsequently ligated together to construct the plasmid pUC-X-FKBP-A (Fig. 1).

In addition, the plasmid containing a promoter region of natural GAPDH gene (pGXN13-NeoC-ATE; Japanese Patent Application No. 1-328264) is double-digested with *Xho*I and *Bam*HI, and a large fragment obtained is ligated with a smaller fragment (containing HSA cDNA) of *Xho*I/*Bam*HI double digests of the expression plasmid pJDB-ADH-HSA-A which contains human serum albumin cDNA (see Japanese Patent Application Laying-Open (KOKAI) No. 2-117384). The resulting pJDB-GAP-HSA-A is then double-digested with *Sma*I and *Xho*I to obtain an expression vector from which the HSA gene is removed. Separately from this, pUC-X-FKBP-A is in turn subjected to *Hind*III digestion, the treatment with Klenow fragment and *Xho*I digestion to obtain a 570 bp DNA fragment containing FKBP and poly A sequences. By ligating these DNA fragments with each other, the expression plasmid pJDB-GAP-FKBP-A is constructed for the expression and production of human FKBP (see Fig. 2). As illustrated by the structure of the expression plasmid in Fig. 2, a DNA fragment encoding human FKBP is introduced into a site downstream from the GAPDH promoter and upstream from the ADH I terminator. The expression plasmid pJDB-GAP-FKBP-A has been transferred into *E. coli* strain HB101, and the transformant obtained has been deposited on June 27, 1990 with Fermentation Research Institute, Agency of Industrial Science and Technology, 1-3 Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305, Japan, as the transformant HB101/pJDB-GAP-h-FKBP (Accession Number: FERM P-11558). This deposition has been subsequently converted on July 4, 1991 to an international deposition under Budapest Treaty by the same depositary institution as an international depositary authority set forth in Budapest Treaty to be given new Accession Number: FERM BP-3471.

Preparation of transformant:

The present invention also provides a transformant obtained by transforming a host with the expression vector of this invention.

The transformant may be prepared by incorporating the expression plasmid pJDB-GAP-FKBP-A into a host by a modification of the KUR method of Hashimoto and Kimura (*Hakko To Kogyo* (Japan), vol.43, pp.630 - 637, 1985). Various microorganisms generally used in the art, for example, *E. coli*, *Bacillus subtilis* and yeast, can be employed as a host in the transformation step. It is apparent to those skilled in the art that animal cells can also be employed as host.

Production of recombinant human FKBP:

Recombinant human FKBP can be obtained by culturing the transformant in an appropriate medium to produce and accumulate the protein. Illustratively, such a process comprises the steps of:

- 5 constructing an expression vector capable of undergoing replication and of expressing a DNA molecule that comprises the base sequence coding for the amino acid sequence of position 1 - position 108 of SEQ ID NO:1, in an appropriate host;
- incorporating said expression vector into said host to produce transformant cells;
- 10 culturing said transformant cells under such conditions that said DNA is expressed to produce said recombinant human FKBP; and
- recovering said recombinant human FKBP.

The process for the manufacture of recombinant human FKBP is also included within the scope of the present invention.

- 15 Detection of the expressed product was carried out by collecting cells from the culture, lysing the cells on heat and analyzing the lysed sample by SDS-polyacrylamide gel electrophoresis, and thereby a single band characterized by human FKBP was detected at about 14 kilo daltons on the gel. The expressed product can be recovered in a substantially purified form, by disrupting the cells and then subjecting the extracts obtained to conventional purification means, for example, a combination of gel filtration, ion exchange chromatography and affinity chromatography. Accordingly, the present invention is also directed
- 20 to the recombinant human FKBP obtained by the process.

- According to the present invention, it is possible to produce the recombinant human FKBP on a large scale using genetic engineering techniques, and the FKBP having PPlase activity will be supplied for the screening of immunosuppressive agents. In addition, the recombinant protein of the present invention may be utilized for activation of an inactive protein, because said recombinant protein is expected to promote
- 25 formation of the higher-order structure of a different substrate protein from that of cyclophilin.

The present invention is further illustrated by the following examples in detail, but it should be understood to those skilled in the art that the present invention is never limited by the examples within the scope of the invention.

30 ExamplesExample 1 Cloning of human FKBP gene

- A human T cell cDNA library, which has been prepared using λ gt11 as a vector, supplied from
- 35 CLONTECH Inc.(U.S.A.) was used to obtain a clone containing human FKBP cDNA.

- 1 $\times 10^4$ pfu of λ gt11 recombinant phages were added to 50 μ l of the culture of *E. coli* strain Y1090 which has been precultured overnight in LB medium (1% tryptone, 0.5% yeast extracts, 0.5% NaCl) supplemented with 0.2% maltose, and the mixture was incubated at 37°C for 20 minutes. The resulting incubation mixture was mixed with 3 ml of L-Top-agarose (LB medium plus 0.7% agarose) and seeded on
- 40 four L-plates (LB medium; 1.5% agar) of 90 mm in diameter. All the plates were cultured overnight at 37°C to form plaques and then maintained at 4°C for 1 hour. Recombinant phage particles were transferred to a membrane filter (Hybond-N, Amersham), and the filter was placed for 5 minutes on a 3MM filter paper (Whatman), which has been pre-soaked in a solution containing 0.5 N NaOH and 1 M NaCl, and then for
- 45 further 5 minutes on another filter paper pre-soaked in 0.5 M Tris-HCl (pH 7.2) plus 1.5 M NaCl. The thus-treated membrane filter was washed with 2 x SSC (20 x SSC consists of 3 M NaCl and 0.3 M trisodium citrate), air-dried, covered with a resin film and then exposed to UV rays in order to fix phage DNA on the filter. Thereafter, the membrane filter was subjected to screening using 32 P-labeled synthetic oligonucleotide (specific activity, $\geq 10^7$ cpm/ μ g DNA) as a probe (Benton and Davis, *Science*, vol.196, pp.180 - 182, 1977).

- The probe DNA fragment 5'-GA(A/G)GA(T/C)GG(G/A/T/C)AA(A/G)AA(A/G)TT(T/C)GA-3' for hybridization,
- 50 which corresponds to a sequence of from glutamic acid at position 31 to aspartic acid at position 37 in the amino acid sequence of bovine FKBP (Harding *et al.*, *Nature*, vol.341, pp.758 - 760, 1989), was synthesized using the automatic DNA synthesizer (Model 380B, Applied Biosystems) with the principle of the phosphoamidite method developed by Caruthers *et al.* (Matteucci, M.D. and Caruthers, M.H., *Tetra-*
- 55 *hedron Letters*, vol.21, p.719, 1980). The 5' end of the DNA chain synthesized (21 pmol) was phosphorylated by treating at 37°C for 60 minutes in 50 μ l of the solution consisting of 50 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 5 mM dithiothreitol, 100 μ Ci of [γ - 32 P] ATP (3,000 Ci/mmol, Amersham) and 12 units of T4 polynucleotide kinase (Takara Shuzo Co., Ltd.). The membrane filter prepared above was subjected to hybridization at 37°C for 16 hours in the solution containing 6 x SSC, 5 x Denhardt's solution

(100 x Denhardt's solution consists of 2% bovine serum albumin, 2% Ficoll and 2% polyvinyl pyrrolidone), 0.5% SDS, 50 µg/ml of ultrasonic-treated salmon sperm DNA, and 10^6 cpm/ml of said probe DNA. The filter was washed with 2 x SSC at 37°C and then exposed to X-ray at -70°C for 10 hours overlaid with an X-ray film (XAR-5, Kodak).

5 After development of the film, each of the two plaques which showed positive signals was scratched off using the tip of a Pasteur pipet and the phage particles obtained were suspended in 100 µl of TM solution [10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂]. After standing still at room temperature for 20 minutes, 0.5 µl of the suspension was diluted with 1 ml of TM solution. *E. coli* Y1090 cells were infected with 5 µl of the suspension diluted, seeded on L-plate, and cultured in the same manner as above in order to form plaques.

10 By subjecting the plaques formed to plaque hybridization in the same manner as described above, a positive clone forming a single plaque was harvested. The positive clone was scratched off using the tip of a Pasteur pipet, added to 50 µl of the suspension of *E. coli* strain Y1090 and maintained at 37°C for 20 minutes. The resulting mixture was then added to 2 ml of LB medium containing 10 mM MgSO₄ and cultured at 37°C for 6 hours with shaking. The culture obtained was mixed with 100 µl of chloroform, stirred

15 on a Vortex mixer to lyse the cells completely and then subjected to centrifugation at 5,000 rpm for 5 minutes. The supernatant contained phage particles of the order of 10^{10} . The supernatant of 800 µl was mixed thoroughly with 100 µl of 5 M NaCl and stood for 20 minutes at -20°C. After centrifuging the mixture at 15,000 rpm for 5 minutes, pellet obtained was washed with 500 µl of 70% ethanol and then dissolved in 200 µl of TE solution [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. The resulting solution was mixed with 1 µl

20 (60 units/µl) of DNase I (Takara Shuzo Co., Ltd.) and 2 µl of 1 M MgCl₂, incubated at 37°C for 30 minutes, and then mixed with 100 µl of TE-saturated phenol on a Vortex mixer. The mixture treated was centrifuged at 12,000 rpm for 5 minutes, and the aqueous layer was extracted once with phenol/chloroform (1:1). To the aqueous layer after extraction was added 20 µl of 3 M sodium acetate (pH 5.2) and 500 µl of ethanol, and the mixture was then centrifuged to precipitate DNA. The precipitate was washed with 70% ethanol, dried *in vacuo* and then dissolved in 50 µl of TE solution. In this manner, about 1 µg of phage DNA was obtained.

25 To 20 µl of the prepared solution were added 2.5 µl of 10 times-concentrated *Eco*RI buffer [0.5 M NaCl, 1 M Tris-HCl (pH 7.5), 70 mM MgCl₂], 1 µl (20 units) of *Eco*RI (Nippon Gene), and 1 µl of 10 mg/ml solution of RNase A (Sigma). After incubating at 37°C for 1 hour, the reaction mixture was subjected to 0.7% agarose gel electrophoresis, and the DNA bands were transferred to a Hybond filter according to the

30 Southern blotting (Southern, E., *J. Mol. Biol.*, vol.98, pp.503 - 517, 1975). Thereafter, hybridization on the DNA-bound filter was carried out under the same conditions as in the plaque hybridization. Each of the clones thus obtained contained an *Eco*RI fragment of about 1.5 kilo bases which was then isolated from agarose gel by glass powder method (Gene Clean™, Bio-101) and subcloned into pUC19 vector.

*Eco*RI-digested pUC19 vector (30 ng) and the 1.5 kb *Eco*RI fragment (20 ng) recovered were treated at

35 16°C for 4 hours in 30 µl of the reaction mixture [66 mM Tris-HCl (pH 7.6), 6.6 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP] containing 2.8 units of T4 DNA ligase (Takara Shuzo Co., Ltd.) so as to obtain a recombinant plasmid in which said digested vector and said 1.5 kb fragment were ligated together. This reaction mixture of 10 µl was used to transform *E. coli* strain JM107 as a host. A competent *E. coli* strain for transformation was prepared by the calcium chloride method (Mandel, M. and Higa, A., *J. Mol. Biol.*, vol.53, pp.159 - 162, 1970). An overnight culture (in LB medium) of *E. coli* strain JM107 was diluted with the

40 same medium and cultured at 37°C with shaking until absorbance at OD₆₆₀ reached 0.6. The culture (1.5 ml) was centrifuged at 5,000 rpm for 5 minutes. The cells collected were suspended in 750 µl of 50 mM CaCl₂ solution, maintained for 20 minutes on ice, and then centrifuged to collect cells. The cells were again suspended in 100 µl of 50 mM CaCl₂ solution, and the cell suspension was mixed with the aforementioned

45 DNA ligase reaction mixture and then stood for 40 minutes on ice. After keeping at 42°C for 1 minute, the resulting mixture was mixed with 1 ml of LB medium and incubated at 37°C for 30 minutes. The mixture (0.1 ml) incubated was applied on an X-Gal plate (L-plate supplemented with 155 µg/ml of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 80 µg/ml of isopropyl-β-D-thiogalactopyranoside and 25 µg/ml of ampicillin). After culturing overnight at 37°C, white colonies were selected from colonies formed on the

50 plate and a loopful of cells of the selected colony were transferred to LB medium supplemented with 25 µg/ml of ampicillin in order to prepare an overnight culture. Cells were collected from 1.5 ml of the overnight culture by centrifugation and then subjected to Mini-Prep of plasmid DNA in the conventional way (Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, 1982). Plasmid DNA obtained was digested with *Eco*RI and the resulting fragments were subjected to agarose gel electrophoresis to confirm the existence

55 of the 1.5 kb *Eco*RI fragment inserted into the pUC vector (pUC-h-FKBP). Binding of the insert to the probe was also confirmed by Southern blotting. At *Hinc*II recognition site located in the DNA insert, 1 µg of the plasmid DNA was double-digested at 37°C for 60 minutes in 25 µl of the reaction mixture [50 mM NaCl, 100 mM Tris-HCl (pH 7.5), 7 mM MgCl₂, 20 units of *Eco*RI (Nippon Gene) and 10 units of *Hinc*II (Nippon

Gene]). Each of the two DNA fragments (about 750 bp each) formed was inserted into M13mp phage DNA so as to determine their nucleotide sequences by the dideoxy method (Sanger, F., Nicklen, S. and Corlson, A.R., *Proc. Natl. Acad. Sci. USA*, vol.74, pp.5463 - 5467, 1977). In this way, the cDNA which encodes human FKBP was identified. The determined nucleotide sequence of the 1.5 kb *EcoRI* fragment is shown in SEQ ID NO:1 of Sequence Listing. This fragment consists of 1532 base pairs. By comparing the deduced amino acid sequence from the base sequence with the partial amino acid sequence of bovine FKBP reported by Harding *et al.* (*Nature*, vol.341, pp.758 - 760, 1989), it was revealed that this inventive gene encodes human FKBP consisting of 108 amino acids of from position 1 to position 108 represented by SEQ ID NO:1.

Example 2 Cloning of human cyclophilin cDNA

A human T cell cDNA library, which has been prepared using λ gt11 as a vector, supplied from CLONTECH Inc.(U.S.A.) was used to obtain a clone which contained human cyclophilin cDNA. *E. coli* strain Y1090 as a host was infected with λ gt11 recombinant phages and thus 1×10^4 of plaques were formed on an L-plate, and the recombinant DNA fragments obtained were transferred to a membrane filter (Hybond-N, Amersham). The membrane filter was then screened using ^{32}P -labeled porcine PPlase cDNA gene (specific activity, $\geq 10^8$ cpm/ μg) as a probe (Benton and Davis, *Science*, vol.196, pp.180 - 182, 1977).

The probe was prepared by labeling the *EcoRI*/*PstI* fragment of the porcine PPlase gene as disclosed in Japanese Patent Application No. 1-184738 (filed July 19, 1989) using a randomly primed DNA labeling kit (Boehringer) according to the supplier's manual attached to the kit. The membrane filter prepared above was subjected to hybridization at 65°C for 16 hours in the solution consisting of $4 \times \text{SSC}$, $5 \times$ Denhardt's solution, 0.5% SDS, 50 $\mu\text{g}/\text{ml}$ of ultrasonic-treated salmon sperm DNA and 10^6 cpm/ml of said probe DNA. Thereafter, the filter was washed with $0.1 \times \text{SSC}$ at 65°C and then exposed to X-ray for 10 hours at -70°C overlaid with an X-ray film (XAR-5, Kodak). After development of the film, the plaque which showed a positive signal was subjected repeatedly to the hybridization step until a positive clone was obtained as a single plaque.

Phage DNA was prepared from the positive clone (Blattner *et al.*, *Science*, vol.202, pp.1279 - 1284, 1978), and 1 μg of the phage DNA was digested at 37°C for 60 minutes in 25 μl of the reaction mixture [50 mM NaCl, 100 mM Tris-HCl (pH 7.5), 7 mM MgCl_2 and 20 units of *EcoRI* (Nippon Gene)]. The resulting digests were subjected to Southern blotting (Southern, E., *J. Mol. Biol.*, vol.98, pp.503 - 517, 1975) and the blots on filter were hybridized with said probe. The hybridized *EcoRI* fragment of about 750 bp was recovered from the agarose gel by the glass powder method (Gene CleanTM, Bio-101) and subcloned into pUC19 vector.

EcoRI-digested pUC19 vector (30 ng) and the *EcoRI* fragment (determined to be 800 bp) (20 ng) recovered were treated at 16°C for 4 hours in 30 μl of the reaction mixture [66 mM Tris-HCl (pH 7.6), 6.6 mM MgCl_2 , 10 mM dithiothreitol, 1 mM ATP] containing 2.8 units of T4 DNA ligase (Takara Shuzo Co., Ltd.) to obtain a recombinant plasmid in which said digested vector and said 800 bp fragment were ligated together. *E. coli* strain JM107 was transformed with 10 μl of the reaction mixture obtained, and the transformant was inoculated on the X-Gal plate in order to form colonies. A white colony was selected and then inoculated in 5 ml of LB medium containing 25 $\mu\text{g}/\text{ml}$ of ampicillin. After culturing overnight at 37°C , cells were collected from 1.5 ml of the overnight culture by centrifugation and subjected to the Mini-Prep so as to prepare DNA. After cleavage of the DNA with *EcoRI*, the fragments were subjected to agarose gel electrophoresis to obtain the recombinant plasmid pUC-h-PPI containing an appropriate DNA insert.

At the *PstI* recognition site located in the DNA insert, 1 μg of the plasmid DNA was double-digested at 37°C for 60 minutes in 25 μl of the solution consisting of 50 mM NaCl, 100 mM Tris-HCl (pH 7.5), 7 mM MgCl_2 , 20 units of *EcoRI* (Nippon Gene) and 20 units of *PstI* (Nippon Gene). About 600 bp- and about 150 bp-DNA fragments formed were separately inserted into a M13mp phage DNA in order to determine their nucleotide sequences by the dideoxy method (Sanger, F., Nicklen, S. and Corlson, A.R., *Proc. Natl. Acad. Sci. USA*, vol.74, pp.5463 - 5467, 1977). The cDNA sequence of human cyclophilin has already been determined and reported (Haendler, B *et al.*, *ENBO J.*, vol.6, pp.947 - 950, 1987). The nucleotide sequence of the clone obtained completely coincided with the reported sequence.

Example 3 Construction of human FKBP expression plasmid for use in yeast

One(1) μg of the plasmid pUC-h-FKBP DNA containing FKBP cDNA was digested in 20 μl of the reaction mixture [150 mM NaCl, 6 mM Tris-HCl (pH 7.5), 6 mM MgCl_2 and 6 units of *NcoI* (Nippon Gene)] at 37°C for 1 hour. The digests were mixed with 80 μl of TE and heated at 70°C for 5 minutes. To the

heat-treated sample was added 1 μ l of 1 mM dXTP (dATP, dGTP, dCTP or dTTP) and 2 units of DNA polymerase Klenow fragment (Takara Shuzo Co., Ltd.), and the mixture was incubated at 37°C for 30 minutes. The resulting reaction mixture was subjected to agarose gel electrophoresis, and a 340 bp DNA fragment blunt-ended which has a *Nco*I site was isolated from the gel and purified using Gene Clean™.

5 Separately from this, 1 μ g of the cloning vector pUC18X in which a *Xho*I site has been introduced into the *Eco*RI site of pUC18 was double-digested in 20 μ l of the reaction mixture [20 mM KCl, 10 mM Tris-HCl (pH 8.0), 7 mM MgCl₂, 20 units of *Eco*RI (Nippon Gene) and 10 units of *Sma*I (Takara Shuzo Co., Ltd.)] at 37°C for 1 hour. The digests were mixed with 20 μ l of 40 mM Tris-HCl (pH 7.5) and heated at 70°C for 5 minutes. Thereafter, Klenow treatment was carried out in the same manner as described above to obtain a
10 blunt-ended vector. This vector (30 ng) and the aforementioned FKBP-containing DNA fragment (20 ng) were treated at 16°C for 10 hours in 30 μ l of the ligation mixture [66 mM Tris-HCl (pH 7.6), 6.6 mM MgCl₂, 10 mM dithiothreitol and 1 mM ATP] containing 2.8 units of T4 DNA ligase (Takara Shuzo Co., Ltd.) so as to obtain the recombinant plasmid pUC-X-FKBP in which said vector were ligated with said DNA fragment.

To 20 μ l of the *Eco*RI reaction mixture [50 mM NaCl, 100 mM Tris-HCl (pH 7.5), 7 mM MgCl₂ and 20
15 units of *Eco*RI] was added 1 μ l of *Pst*I (20 units, Nippon Gene), and digestion of the plasmid pUC-h-PPI DNA (1 μ g) containing the human cyclophilin cDNA was carried out in said mixture solution at 37°C for 1 hour to obtain an about 150 bp DNA fragment containing the human cyclophilin poly A sequence. In the same manner, a DNA fragment containing a 370 bp FKBP gene was obtained from the plasmid pUC-X-FKBP by double-digesting 1 μ g of the plasmid DNA at 37°C for 1 hour in 20 μ l of the *Eco*RI reaction
20 mixture which contains 1 μ g of *Xho*I (12 units, Takara Shuzo Co., Ltd.) and 1 μ g of *Pst*I (20 units). As a cloning vector, a 2.6 kb-vector fragment was obtained by digesting 200 ng of the pUC18X DNA at 37°C for 1 hour in a mixture mixture of 20 μ l of the *Eco*RI reaction mixture and 1 μ l of *Xho*I (12 units).

Thereafter, the plasmid pUC-X-FKBP-A was constructed in which these three DNA fragments were ligated together (see, Fig. 1).

25 Separately from this, the plasmid containing a promoter region of the intact GAPDH gene (pGXN13-NeoC-ATE; Japanese Patent Application No. 1-328624) was double-digested with *Xho*I and *Bam*HI, and a large fragment obtained was ligated with a smaller fragment (containing HSA cDNA) of *Xho*I/*Bam*HI double digests of the expression plasmid pJDB-ADH-HSA-A which contained human serum albumin cDNA (Japanese Patent Application No. 2-117384). One(1) μ g of pJDB-GAP-HSA-A DNA thus prepared was then
30 double-digested at 37°C for 1 hour in 20 μ l of the reaction mixture [20 mM KCl, 10 mM Tris-HCl (pH 8.0), 7 mM MgCl₂, 10 units of *Sma*I and 12 units of *Xho*I] to obtain an expression vector from which the HSA gene was removed.

From the plasmid pUC-X-FKBP-A, a 570 bp-DNA fragment containing FKBP and poly A sequences was obtained in the following manner: One(1) μ g of the plasmid DNA was digested at 37°C for 1 hour in the
35 reaction mixture [50 mM NaCl, 100 mM Tris-HCl (pH 7.5), 7 mM MgCl₂ and 10 units of *Hind*III (Takara Shuzo Co., Ltd.)]. Thirty μ l of water were added to the reaction mixture and heated at 70°C for 5 minutes. The heat-treated sample was subjected to the treatment with Klenow fragment and then again to heat treatment. Thereafter, the resulting sample was mixed with 5 μ l of 1 M NaCl and 1 μ l (12 units) of *Xho*I, and the mixture was incubated at 37°C for 1 hour to obtain a 570 bp-DNA fragment.

40 By ligating these DNA fragments, the expression plasmid pJDB-GAP-FKBP-A was constructed for use in yeast cells (see Fig. 2).

Example 4 Transformation of yeast host cells with expression plasmid

45 Transformation of yeast cells with the human FKBP expression plasmid pJDB-GAP-FKBP-A was carried out by a modification of the KUR method reported by Hashimoto and Kimura (*Hakko To Kogyo* (Japan), vol.43, pp.630 - 637, 1985). The yeast strain AH22 (MATa, *leu* 2-3, *leu* 2-112, *his* 4-519, *can1*) was cultured overnight in YPD medium [1% Yeast extracts (Difco Laboratories), 2% Bacto Pepton (Difco Laboratories) and 2% glucose]. One(1) ml of the overnight culture was inoculated in 50 ml of YPD medium
50 and cultured at 30°C until absorbance at OD₆₀₀ reached 0.5. The culture kepted at 4°C was then centrifuged at 2,000 rpm for 5 minutes, and the cells collected were resuspended in 5 ml of 0.1 M LiSCN. 1.5 ml aliquot of the cell suspension was centrifuged at 2,000 rpm for 5 minutes and the cells collected were resuspended in the solution consisting of 10 μ l of 2 M LiSCN and 46 μ l of 50% polyethylene glycol 4000. To the suspension was then added 10 μ l of the DNA solution containing the plasmid DNA of 5 to 10
55 μ g, and the mixture was incubated overnight at 30°C. The suspension was mixed with 50 μ l of sterile distilled water, stirred gently on a Vortex mixer, and subsequently centrifuged at 2,000 rpm for 5 minutes. The cells collected were resuspended in 100 μ l of sterile distilled water and seeded on the selection agar medium [SD medium: 20 μ g/ml of histidine hydrochloride, 0.67% amino acid-free Yeast Nitrogen Base

(Difco Laboratories) and 2% glucose, supplemented with 2% agar]. Several days after cultivation at 30 °C, each colony formed was inoculated in 5 ml of SD medium and cultured at 30 °C for 48 hours in order to examine the expression of human FKBP by means of SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

5

Example 5 Expression of human FKBP by transformant

One(1) ml of the culture obtained by culturing the transformant in SD medium for 48 hours was centrifuged at 5,000 rpm for 5 minutes, and the cells collected were resuspended in 30 µl of the buffer solution for SDS-PAGE sample (2% SDS, 5% 2-mercaptoethanol, 7% glycerol, 0.00625% Bromophenol Blue and 0.0625 M Tris-HCl pH 6.8). After boiling the cell suspension at 100 °C for 10 minutes, 10 µl of the resulting sample was subjected to electrophoresis on an SDS-polyacrylamide gel with 15% of separation gel concentration (Laemmli's method: *Nature*, vol.277, p.680, 1970). At the end of the electrophoresis, the gel was stained with Coomassie Brilliant Blue (CBB), a single band corresponding to human FKBP was detected at about 14 kilo daltons of molecular weight.

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Sequence Listing

5

SEQ ID NO: 1

SEQUENCE LENGTH: 1532

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SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

15

IMMEDIATE SOURCE

Human T cell cDNA library (ex CLONTECH Inc., USA)

FEATURE

20

FEATURE KEY: mat peptide

LOCATION: 79 402

IDENTIFICATION METHOD: S

25

SEQUENCE DESCRIPTION

EcoRI

30

GAATTCGGGC CGCCGCCAGG TCGCTGTTGG TCCACGCCGC CCGTCGCGCC GCCCGCCCGC 60

TCAGCGTCCG CCGCCGCC ATG GGA GTG CAG GTG GAA ACC ATC TCC CCA GGA 111

Met Gly Val Gln Val Glu Thr Ile Ser Pro Gly

1

5

10

35

GAC GGC CGC ACC TTC CCC AAG CGC GGC CAG ACC TGC GTG GTG CAC TAC 159

Asp Gly Arg Thr Phe Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr

15

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25

40

ACC GGC ATG CTT GAA GAT GGA AAG AAA TTT GAT TCC TCC CGG GAC AGA 207

Thr Gly Met Leu Glu Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg

30

35

40

45

AAC AAG CCC TTT AAG TTT ATG CTA GGC AAG CAG GAG GTG ATC CGA GGC 255

Asn Lys Pro Phe Lys Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly

45

50

55

50

TGG GAA GAA GGC GTT GCC CAG ATG AGT GTG GGT CAG AGA GCC AAA CTG 303

Trp Glu Glu Gly Val Ala Gln Met Ser Val Gly Glu Arg Ala Lys Leu

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	60	65	70	75	
	ACT ATA TCT CCA GAT TAT GCC TAT GGT GCC ACT GGG CAC CCA GGC ATC				351
5	Thr Ile Ser Pro Asp Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile				
		80	85	90	
	ATC CCA CCA CAT GCC ACT CTC GTC TTC GAT GTG GAG CTT CTA AAA CTG				399
	Ile Pro Pro His Ala Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu				
10		95	100	105	
	GAA TGA CA GGAATGGCCT CCTCCCTTAG CTCCCTGTTC TTGGATCTGC CATGGAGGGA				457
	Glu				
	TCTGGTGCCT CCAGACATGT GCACATGAGT CCATATGGAG CTTTTCCTGA TGTTCCTCTC				517
15	CACCTTGTAT AGACATCTGC CCTGACTGAA TGTGTTCTGT CACTCAGCTT TGCTTCCGAC				577
	ACCTCTGTTT CCTCTTCCCC TTTCTCCTCG TATGTGTGTT TACCTAACT ATATGCCATA				637
	AACCTCAAGT TATTCATTTT ATTTTGTMTT CATTTTGGGG TGAAGATTCA GTTTCAGTCT				697
20	TTTGGATATA GGTTTCCAAT TAAGTACATG GTCAAGTATT AACAGCACAA GTGGTAGGTT				757
			SspI		
	AACATTAGAA TAGGAATTGG TGTGTTTTGGG GGGGTTTGCA AGAATATTTT ATTTTAATTT				817
	TTTGGATGAA ATTTTTATCT ATTATATATT AACATTCTT GCTGCTGCGC TGCAAAGCCA				877
25	TAGCAGATTT GAGGCGCTGT TGAGGACTGA ATTACTCTCC AAGTTGAGAG ATGTCCTTGG				937
	GTTAAATTAA AAGCCCTACC TAAAACTGAG GTGGGGATGG GGAGAGCCTT TGCCTCCACC				997
	ATTCCCCACC ACCCTCCCCT TAAACCCTCT GCCTTTGAAA GTAGATCATG TTCCTGCAA				1057
30	TGCTGGACAC TACAGGTATC TGTCCCTGGG CCAGCAGGGA CCTCTGAAGC CTTCTTTGTG				1117
	ATAACTTTCC AAGCTCCACC ACTTCCTAAA TCTTAAGAAC TTTAATTGAC AGTTTCAATT				1237
	GAAGGTGCTG TTTGTAGACT TAACACCCAG TGAAAGCCCA GCCATCATGA CAAATCCTTG				1297
	AATGTTCTCT TAAGAAAATG ATGCTGGTCA TCGCAGCTTC AGCATCTCCT GTTTTTTGAT				1357
35	GCTTGGCTCC CTCGCTGAT CTCAGTTTCC TGGCTTTTCC TCCCTCAGCC CCTTCTCACC				1417
	CCTTTGCTGT CCTGTGTAGT GATTGGTGA GAAATCGTTG CTGCACCCCT CCCCAGCAC				1477
			EcoRI		
40	CATTTATGAG TCTCAAGTTT TATTATTGCA ATAAAGTGC TTTATGCCCG AATTC				1532

Claims

1. A DNA molecule comprising a base sequence coding for human FK506-binding protein which is represented by the following amino acid sequence:

EP 0 594 847 A1

1	5	10
Met Gly Val Gln Val Glu Thr Ile Ser Pro		
5		
	15	20
Gly Asp Gly Arg Thr Phe Pro Lys Arg Gly		
10		
	25	30
Gln Thr Cys Val Val His Tyr Thr Gly Met		
15		
	35	40
Leu Glu Asp Gly Lys Lys Phe Asp Ser Ser		
20		
	45	50
Arg Asp Arg Asn Lys Pro Phe Lys Phe Met		
25		
	55	60
Leu Gly Lys Gln Glu Val Ile Arg Gly Trp		
30		
	65	70
Glu Glu Gly Val Ala Gln Met Ser Val Gly		
35		
	75	80
Glu Arg Ala Lys Leu Thr Ile Ser Pro Asp		
40		
	85	90
Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly		
45		
	95	100
Ile Ile Pro Pro His Ala Thr Leu Val Phe		
50		
	105	
Asp Val Glu Leu Leu Lys Leu Glu		
55		

2. A DNA molecule according to claim 1 wherein said base sequence comprises the following sequence:

	ATG GGA GTG CAG GTG GAA ACC ATC TCC CCA	30
5	GGA GAC GGG CGC ACC TTC CCC AAG CGC GGC	60
	CAG ACC TGC GTG GTG CAC TAC ACC GGG ATG	90
10	CTT GAA GAT GGA AAG AAA TTT GAT TCC TCC	120
	CGG GAC AGA AAC AAG CCC TTT AAG TTT ATG	150
	CTA GGC AAG CAG GAG GTG ATC CGA GGC TGG	180
15	GAA GAA GGG GTT GCC CAG ATG AGT GTG GGT	210
	CAG AGA GCC AAA CTG ACT ATA TCT CCA GAT	240
20	TAT GCC TAT GGT GCC ACT GGG CAC CCA GGC	270
	ATC ATC CCA CCA CAT GCC ACT CTC GTC TTC	300
	GAT GTG GAG CTT CTA AAA CTG GAA	324
25		

3. An expression vector containing the DNA molecule according to claim 1 or claim 2, wherein said DNA molecule is introduced into a cloning site downstream from a promoter and upstream from a terminator in the vector.
4. An expression vector according to claim 3 wherein said vector is a plasmid.
5. An expression vector according to claim 4 wherein said vector is pJDB-GAP-FKBP-A.
6. A transformant obtained by transforming a host with the expression vector according to any one of claims 3 to 5.
7. A transformant according to claim 6 wherein said host is yeast or *Escherichia coli*.
8. A process for manufacturing a recombinant human FK506-binding protein that comprises the amino acid sequence encoded by the base sequence according to claim 1 or claim 2, which comprises the steps of:
 constructing an expression vector capable of replicating and of expressing a DNA molecule containing said base sequence in an appropriate host;
 incorporating said expression vector into the host to produce transformant cells;
 culturing said transformant cells under such conditions that said DNA is expressed to produce said recombinant human FK506-binding protein; and
 recovering said recombinant human FK506-binding protein.
9. A recombinant human FK506-binding protein comprising the amino acid sequence encoded by the base sequence according to claim 1 or claim 2.

Fig. 1

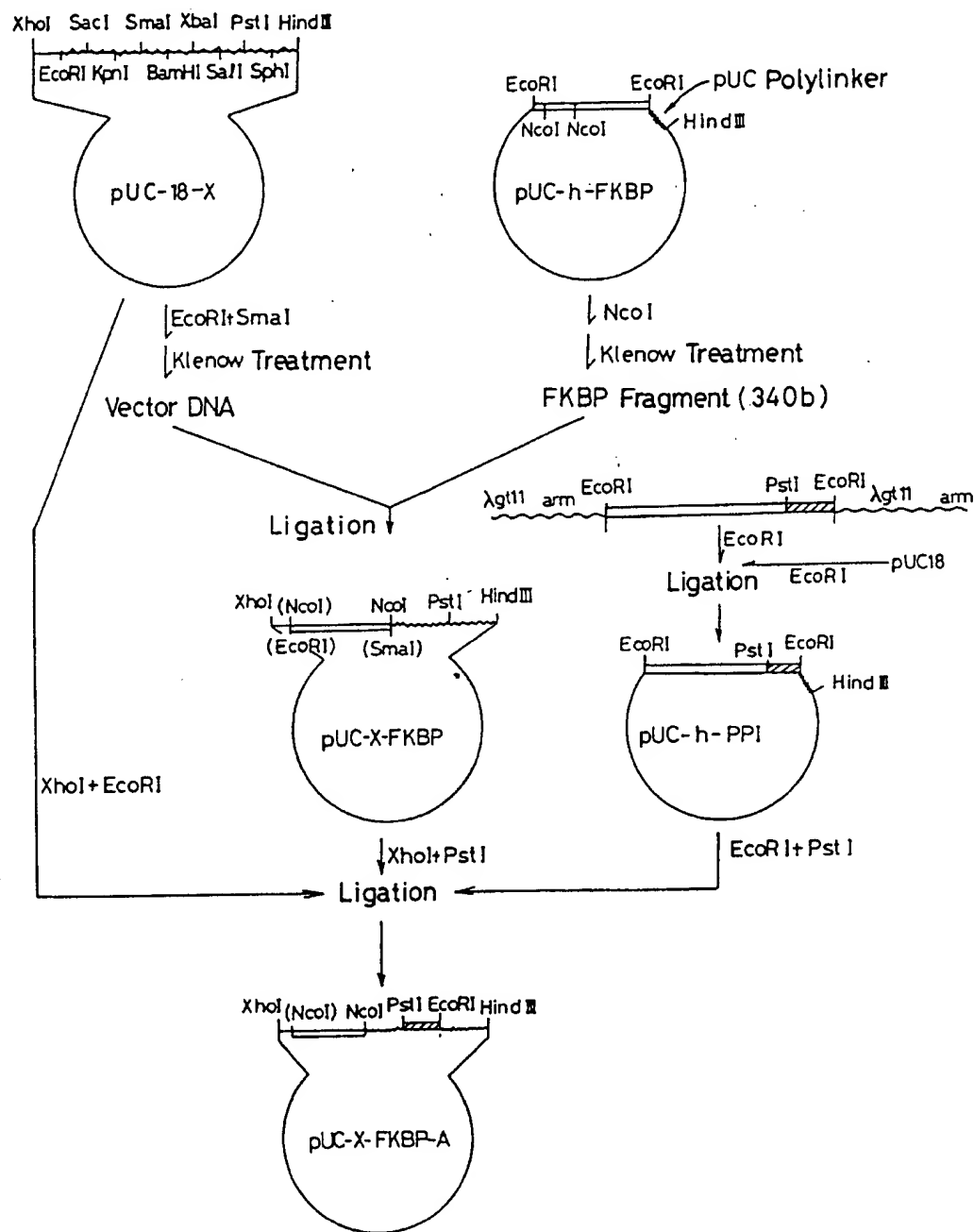
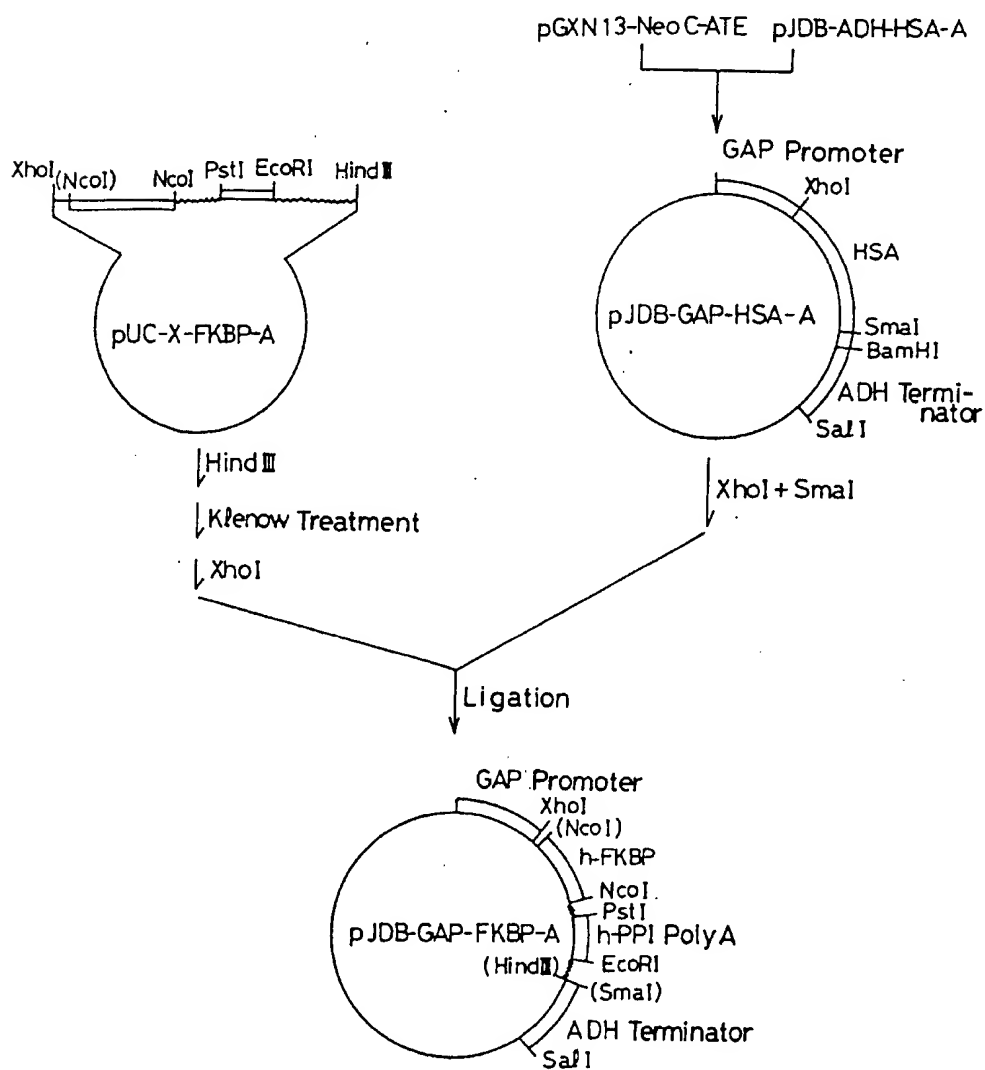


Fig. 2



INTERNATIONAL SEARCH REPORT

International Application No PCT/JP91/00931

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
C12N15/12, 15/81, 1/19, 1/21, C12P21/02, C07K13/00 // (C12N1/19, C12R1:645) (C12N1/21, C12R1:19), (C12P21/02, C12R1:645)		Int. Cl ⁵
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC	C12N15/00-15/90, C12N1/19, C12N1/21, C12P21/00-21/02, C07K13/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
Biosis Database, Chemical Abstracts Database (CA, REG), EMBL-GDB, Gen Bank, NBRF-PDB, Swiss-Prot		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X/Y	Proc. Natl. Acad. Sci. USA, Vol.87, No.14, 1990, Maki N. et al. "Complementary DNA encoding the human T-cell FK-506-binding protein, a peptidylprolyl cis-trans isomerase distinct from cyclophilin" p.5440-5443	1-7/8
Y	Sambrook et al. "Molecular Cloning" 1989, Cold Spring Harbor Laboratory Press, Section 16-17	8
X	Nature, Vol.341, 1989, Siekierka J. et al. "A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin" p.755-757	9
X	Nature, Vol.341, 1989, Harding M. et al. "Areceptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase" p.758-760	9
<p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"8" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
October 7, 1991 (07. 10. 91)	October 21, 1991 (21. 10. 91)	
International Searching Authority	Signature of Authorized Officer	
Japanese Patent Office		